EPA/OPP MICROBIOLOGY LABORATORY
ESC, Ft. Meade, MD

Standard Operating Procedure
for
Sterility Assessment of Disinfectant Product Samples

SOP Number: QC-18-02

Date Revised: 09-10-02

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Controlled Copy No.: _____

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1.0 SCOPE AND APPLICATION:

1.1 This protocol describes quality control practices that may be performed on disinfectant product samples to assess their sterility.

2.0 DEFINITIONS: None

3.0 HEALTH AND SAFETY:

3.1 Disinfectants may contain a number of different active ingredients, such as heavy metals, aldehydes, peroxides, phenol, etc. Latex gloves and other personal protective clothing or devices are worn during the handling of these items. A chemical fume hood or other containment equipment is employed when performing tasks with concentrated products.

4.0 CAUTIONS: None

5.0 INTERFERENCES:

5.1 Aseptic procedures must be followed during this assay to avoid accidental contamination of the product. Exposing the product to external contaminants during opening and dispensing, and the use of non-sterile laboratory supplies may interfere with the outcome of this analysis. Quality control measures for media, reagents and pre-sterilized supplies used in this evaluation must be followed as outlined in SOP QC-11, Performance and Sterility of Media and Reagents, and SOP QC-12, Sterility of Pre-Sterilized and Autoclaved Supplies.

5.2 Cloudiness of culture media due to the interaction of the disinfectant and media may interfere with the evaluation of the culture tubes. A Gram stain on the cloudy media to verify the presence or absence of microbial growth is performed.

6.0 PERSONNEL QUALIFICATIONS:

6.1 Personnel are required to be knowledgeable about the procedures in this SOP. Documentation of training and familiarization with this SOP can be found in the training file for each employee.

7.0 SPECIAL APPARATUS AND MATERIALS:

7.1 Incubator with temperature reading at the appropriate temperatures, 37±1°C and 55±1°C

7.2 VITEK 32 Identification System

8.0 INSTRUMENT OR METHOD CALIBRATION:

8.1 Refer to the laboratory equipment calibration and maintenance SOPs (SOP EQ series) for details on method and frequency of calibration.

9.0 SAMPLE HANDLING AND STORAGE:

9.1 Disinfectants are stored according to manufacturers' recommendations or at room temperature if the product label or testing parameters do not identify a storage temperature.

10.0 PROCEDURE AND ANALYSIS:

10.1 General Guidelines:

10.1.1 Procedures such as opening the product container, preparing serial dilutions, and inoculating media must be performed under aseptic conditions in a biological safety cabinet.

10.1.2 The sterility assessment should be performed when the product container is initially opened.

10.1.3 Sterility assessments may be performed prior to or concurrently with an efficacy test.

10.1.4 Always follow appropriate chain of custody procedures as stipulated in SOP COC-01, Sample Login and Tracking.

10.1.5 A neutralizer recommended for the product's active ingredient(s) should be used as the diluent (see 10.4). Information on the appropriate neutralizer is included in the product's test parameter.

10.2 Preparation and Opening the Sample Container:

10.2.1 The container must be opened under aseptic conditions in a biological safety cabinet.

10.2.2 For liquids, prior to opening the container, gently shake the container and thoroughly clean the area around the cap and spout with 70% ethanol. Allow the surface to dry. Remove the cap. Do not touch the inside surface of the cap. If present, carefully remove the seal attached to the lip of the spout with autoclaved or cooled, flame-sterilized instruments (i.e., razor blade, forceps).

10.2.3 For spray products, shake the container at least 25 times immediately prior to assay. Remove cap and clean the nozzle and top of can with 70% alcohol. Allow the surface to dry. Don sterile gloves. Spray the product for 10-15 seconds prior to collection of sample.

10.2.4 For towelette products, clean the dispenser or packaging with 70% alcohol. Allow the surface to dry. Aseptically remove a towelette by wearing sterile latex gloves and with the use of sterile (autoclaved or cooled, flame-sterilized) forceps.

10.3 Collection of the Sample:

10.3.1 For liquids, pour approximately 10 mL of the sample into a sterile beaker. Do not place a pipette or any other instrument inside the product container. Place cap on the product container and secure tightly. Initiate serial dilutions from this sample (see 10.4).

10.3.2 For a spray product, spray the product into a sterile beaker for 20-30 seconds. Allow the product to settle (10-15 minutes). Approximately 10 mL of liquid should be collected by this method. Initiate serial dilutions from this sample (see 10.4). When collecting the sample (i.e., spraying), hold

the spray can in one hand, perpendicular to the biological safety cabinet surface. Hold the beaker in the other hand, positioning it so that it is parallel to the biological safety cabinet surface with the open end facing the nozzle of the spray can. The potential for contamination of the sample (i.e., contact of product with sprayer's gloved hand) is reduced as compared to positioning the spray can directly over the beaker (on biological safety cabinet surface) and spraying down into the beaker.

10.3.3 For a towelette, if saturated, carefully express the liquid from a single towelette by squeezing out the liquid into a sterile beaker. Use sterile (autoclaved or cooled, flame-sterilized) forceps to manipulate the towelette. Collect approximately 10 mL of the liquid. More than one towelette may be required to collect a 10 mL sample. Initiate serial dilutions from this sample (see 10.4).

If the towelette is not saturated with liquid, carefully place a folded towelette into 20 mL of letheen broth (38 mm x 100 mm tube) or other suitable neutralizer. Gently agitate the tube containing the towelette. Carefully extract the towelette with sterile (autoclaved or cooled, flame-sterilized) forceps. Express the liquid during the extraction. Initiate serial dilutions from the residual mixture remaining in the tube (see 10.4).

10.3.4 A preparation number must be given to the sample. Fill in information on a Media Preparation form as stipulated in SOP QC-15, Media Prep and Sterilization Run Numbers. In addition, fill in appropriate information on the Sterility Assessment of Product Sample Record (see 16.1).

10.4 Preparation of Serial Dilutions:

10.4.1 Prepare a serial dilution by pipetting 1 mL of the sample into a 9 mL tube of diluent. Prepare dilutions of 1×10^{-1} through 1×10^{-5}. Vortex each dilution tube prior to a transfer. Include a tube of undiluted sample (approx. 5 mL in a 20

mm x 150 mm tube) in the dilution set.

10.5 Inoculation of Culture Media:

10.5.1 Label the media tubes to correspond with the appropriate dilution. Inoculate 10 mL tubes (20 mm x 150 mm) of letheen broth and fluid thioglycollate medium in duplicate with 1 mL of each dilution; include the undiluted sample as well. Thus, a total of 24 tubes (12 letheen broth, 12 fluid thioglycollate medium) will be inoculated per sample.

10.5.2 Include one tube of letheen broth and fluid thioglycollate medium as uninoculated controls.

10.5.3 Incubate tubes at 37±1°C for at least 48 hours. Proceed with section 10.6.

10.5.4 Once results have been read and recorded following incubation at 37±1°C, incubate tubes at 55±1°C for at least 48 hours. Proceed with section 10.6 again.

10.6 Results and Confirmation:

10.6.1 Read tubes of fluid thioglycollate medium before shaking as well as after. Each tube is shaken prior to recording results to determine the presence or absence of turbidity. Report results as + (growth) or 0 (no growth) on the Sterility Assessment of Product Sample Record (see 16.1). A positive result is one in which microbial growth is observed. A negative result is one in which the broth appears clear.

10.6.1.1 If a tube exhibits cloudiness due to the presence of the disinfectant, record the observation as “NR” (=not readable) on the form. Additionally, perform a Gram stain on the tube to verify the presence or absence of microbial growth. Record Gram stain results on the Worksheet for Recording Gram Stain and Acid Fast Reactions (see 16.2). If cells are observed, note this in the comments section of the Sterility Assessment of

Product Sample Record. From the tube, streak the culture on a plate of TSA for initial isolation. Attempt to identify the contaminant by using the VITEK 32 system (see SOP QC-16, VITEK: Culture Identification Numbers).

10.6.2 Growth from at least one representative positive tube (showing turbidity) from each medium will be Gram stained and streaked on TSA for initial identification and isolation. Record Gram stain results on the Worksheet for Recording Gram Stain and Acid Fast Reactions (see 16.2). Attempt to identify the contaminant by using VITEK analysis. Record confirmation results on the Test Microbe Confirmation Sheet (see 16.3).

11.0 DATA ANALYSIS/CALCULATIONS: None

12.0 DATA MANAGEMENT/RECORDS MANAGEMENT:

12.1 Data will be recorded promptly, legibly, and in indelible ink on the appropriate forms. Completed forms are archived in notebooks kept in locked file cabinets in the file room D217. Only authorized personnel have access to the locked files. Archived data is subject to OPP's official retention schedule contained in SOP ADM-03, Records and Archives.

13.0 QUALITY CONTROL:

13.1 The OPP Microbiology Laboratory conforms to 40CFR Part 160, Good Laboratory Practices. Appropriate quality control measures are integrated into each SOP.

13.2 For quality control purposes, the required information is documented on the appropriate record form(s) (see 16.0).

14.0 NONCONFORMANCE AND CORRECTIVE ACTION:

14.1 No further product testing will be initiated if product contamination is detected. Any product test results for testing done concurrently will factor in the results for contaminated samples.

15.0 REFERENCES: None

16.0 FORMS AND DATA SHEETS:

16.1 Sterility Assessment of Product Sample Record

16.2 Worksheet for Recording Gram Stain and Acid Fast Reactions

16.3 Test Microbe Confirmation Sheet

Sterility Assessment of Product Sample Record
OPP Microbiology Laboratory

PRODUCT INFORMATION		BACKGROUND and PREPARATION NUMBERS	
Confirmed by:_____		Confirmed by:_____	
EPA Reg. No.		Date performed/initials	
Name		Letheen broth prep. No.	
Sample No.		Fluid thioglycollate prep. No.	
Lot No.		Other prep No.:	
Container No.			

RESULTS: Date Recorded/Initials:_____

Dilution of Sample	Letheen Broth (+/0)*				Fluid Thioglycollate Medium (+/0)*			
	Tube 1 @ 37°C	Tube 1 @ 55°C	Tube 2 @ 37°C	Tube 2 @ 55°C	Tube 1 @ 37°C	Tube 1 @ 55°C	Tube 2 @ 37°C	Tube 2 @ 55°C
Undiluted Sample								
10^{-1}								
10^{-2}								
10^{-3}								
10^{-4}								
10^{-5}								
Uninoculated Media Control			NA	NA			NA	NA

* + = growth, 0 = no growth
NR=Not Readable

COMMENTS**

** diluent, tubes selected for confirmation, growth characteristics, additional dates results recorded

Worksheet for Recording Gram Stain and Acid Fast Reactions

OPP Microbiology Laboratory

	Source/Tube No. _____ Results:_____	Source/Tube No. _____ Results:_____	Source/Tube No. _____ Results:_____
	Source/Tube No. _____ Results:_____	Source/Tube No. _____ Results:_____	Source/Tube No. _____ Results:_____
	Source/Tube No. _____ Results:_____	Source/Tube No. _____ Results:_____	Source/Tube No. _____ Results:_____

AF+ =Acid fast positive
GPC =Gram positive cocci
GNR =Gram negative rod

Test Microbe Confirmation Sheet

OPP Microbiology Laboratory

TEST INFORMATION/ Confirmed by:_____			
EPA Reg. No.		Test Date	
Name		Test Organism	
Sample No.		Comments:	

Source: Tube/Plate ID	Date/ Initials	Stain Results*	Media Information			Results		
			Type	Prep. No.	Inc. Time/ Temp.	Date/ Initials	Colony Characteristics	API Test**/VITEK ID (if applicable)

* Record Acid Fast or Gram Stain results as GPC=gram positive cocci, GNR=gram negative rods, AFR=acid fast rods, GPR=Gram positive rods.

** API numerical profile number